Electronic Supplementary Information (ESI) for RSC Advances

Mercaptophenylboronic acid modified gold nanoparticle@silica bubbles for buoyant separation and specific enrichment of glycopeptides

Junjie Hu, Rongna Ma, Fei Liu, Yunlong Chen and Huangxian Ju*

State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, P.R. China

Experimental

Materials and reagents. Horseradish peroxidise (HRP, MW ~ 44 kDa), bovine serum album (BSA, MW ~ 66 kDa), human serum immunoglobulin G (human IgG), trypsin (from bovine pancreas, ⩾10000 BAEE units mg^{-1}), 4-mercaptophenylboronic acid (MPB, 90%), 3-aminopropyltriethoxysilane (APTES), *meta*-aminophenylboronic acid (APB) agarose, ammonium bicarbonate (NH_4HCO_3), DL-dithiothreitol (DTT), iodoacetamide (IAA), trifluoroacetic acid (TFA, ⩾90%) and α-cyano-4-hydroxycinnamic acid (CHCA) were purchased from Sigma-Aldrich Inc. (USA). S60HS silica bubbles (average diameter 30 μm, density 0.6 g cm^{-3}) were purchased from 3M Company (USA). Sulfuric acid (H_2SO_4), chloroauric acid ($HAuCl_4 \cdot 4H_2O$) and trisodium citrate were obtained from Shanghai Reagent Company (Shanghai, China). All these reagents were used as received without further purification. Deionized water was prepared with a Milli-Q water purification system (Millipore, Milford, MA, USA).

Apparatus. Scanning electron microscopic (SEM) images were obtained with a Hitachi S-3000N scanning electron microscope at an acceleration voltage of 10 kV. X-ray photoelectron spectroscopic (XPS) measurements were performed using a PHI5000 Versa Probe spectrometer (ULVAC-PHI, Japan) with an ultra high vacuum generator. Infrared spectra were obtained on a Nicolet NEXUS870 Fourier transform infrared (FT-IR) spectrometer (Madison, WI). The static water contact angles were measured with a contact angle system (OCA30, Dataphysic Instruments GmbH, Germany) using droplets of 1 μL of ultrapure water at room temperature (RT).

Preparation of AuNP-coated silica bubbles (Au@SiBs). Gold nanoparticles (AuNPs) were prepared by quickly adding 3.5 mL of 1% trisodium citrate into a boiling aqueous solution of 0.01% $HAuCl_4$ (100 mL) under magnetic stirring. The average diameter of AuNPs was measured to be around 13 nm. The Au@SiBs were then synthesized according to the previously reported method.[S1] Briefly, 0.2 g silica bubbles were first activated with 5 M H_2SO_4, silanized with 1:10 APTES in methanol overnight, washed extensively in methanol and resuspended in 2 mL deionized water. 200 μL bubble solution was added to a 1.5-mL microcentrifuge tube. After the bubbles rose to the top, the water under the bubbles was removed with a 1-mL syringe. Subsequently, 200 μL of AuNPs was mixed with the bubbles and gently agitated at RT until the mixture changed from deep red to almost clear. Afterwards, the clear solution was removed, and the mixing and agitation procedure was repeated till no colour change of the added AuNPs solution was observed.

Preparation of MPB modified Au@SiBs (MPB-Au@SiBs). The obtained Au@SiBs were resuspended in 2 mL of MPB ethanol solution (10 mM), and gently agitated for 6 h to obtain MPB-Au@SiBs, which were alternately washed with ethanol and water for three times and resuspended in deionized water to a final concentration of 10 μg μL^{-1}. The binding amount of MPB on Au@SiBs could be analysed with liquid chromatography (LC). Briefly, 1 mg of Au@SiBs was added into 2 mL

ethanol solution of 0.5 mg mL^{-1} MPB under agitation to obtain the MPB saturated Au@SiBs. Then 20 μL of the supernatant was diluted to 1 mL with methanol, and 2 μL of the resulting solution was submitted for LC analysis with UV detection at 254 nm, using a Shimadzu LC20 system and Agilent column (Poroshell 120SB-C18, 2.7 μm, 3.0*100 mm, Agilent Technologies, Waldbronn, Germany). The mobile phase was the mixture of 45% methanol and 55% water, and its flow rate was 0.45 mL min^{-1}. The amount of MPB in supernatant could be detected according to the peak areas for obtaining the binding amount of MPB on Au@SiBs.

Protein digestion. HRP was dissolved in 50 mM NH_4HCO_3 at pH 8.3 to a final concentration of 1 mg mL^{-1} and denatured by boiling for 5 min. Then, trypsin was added at an enzyme-to-protein ratio of 1:40 (w/w) and incubated for 16 h at 37 ^oC. Digestion of human IgG or BSA was performed by incubating the mixture of 50 μL of 200 mM DTT and 1 mL of 1 mg mL^{-1} IgG or BSA in 50 mM NH_4HCO_3 solution at 100 ^oC for 5 min. After the mixture cooled down to RT, 40 μL of 1 M IAA was added in dark for 45 min, and the excessive IAA was consumed with 200 μL of 200 mM DTT at RT for 1 h. Then, trypsin was added at an enzyme-to-protein ratio of 1:40 (w/w) and incubated for 16 h at 37 ^oC . The obtained samples were stored at -20 ^oC prior to use.

Glycopeptide enrichment. 10 μL of 10 μg $μL^{-1}$ MPB-Au@SiBs was added into 100 μL HRP tryptic digestions and incubated on a vortex shaker with shaking at RT for 2 h. After the MPB-Au@SiBs were allowed to float to the surface, the solution under the bubbles was carefully removed with a 1-mL syringe, and the left bubbles were washed with NH_4HCO_3 solution to remove the nonspecifically absorbed peptides. The MPB-Au@SiBs were then incubated with 10 μL pH 1.9 solution containing 50% acetonitrile and 1% TFA at RT for 1 h to obtain the sample for mass spectrometric analysis.

Mass spectrometric analysis. Matrix-assisted laser desorption/ionization-time of flight mass spectrometric (MALDI-TOF MS) experiments were performed in a positive ion mode on a 4800

Proteomics Analyzer (Applied Biosystems, USA) with the Nd-YAG laser at 355 nm, a repetition rate of 200 Hz and an acceleration voltage of 20 kV. A saturated solution of CHCA in the mixture of 60% acetonitrile, 0.1% TFA and 39.9% water (V/V/V) was used as matrix. After 1 μL of the sample was deposited on the plate and equal amount of the matrix was introduced, MALDI MS analysis was performed.

Binding capacity of glycopeptides. In order to obtain the binding capacity of glycopeptides to the materials, a series of 100 μg MPB-Au@SiBs were incubated with 1 mL of HRP tryptic digests at different concentrations for 2 h under RT. After the MPB-Au@SiBs floated to the surface, the supernatant was analyzed by MALDI-TOF MS. Only when the total amount of glycopeptides was higher than the binding capacity of the MPB-Au@SiBs, the MS signal was detectable.

Characterization of MPB-Au@SiBs

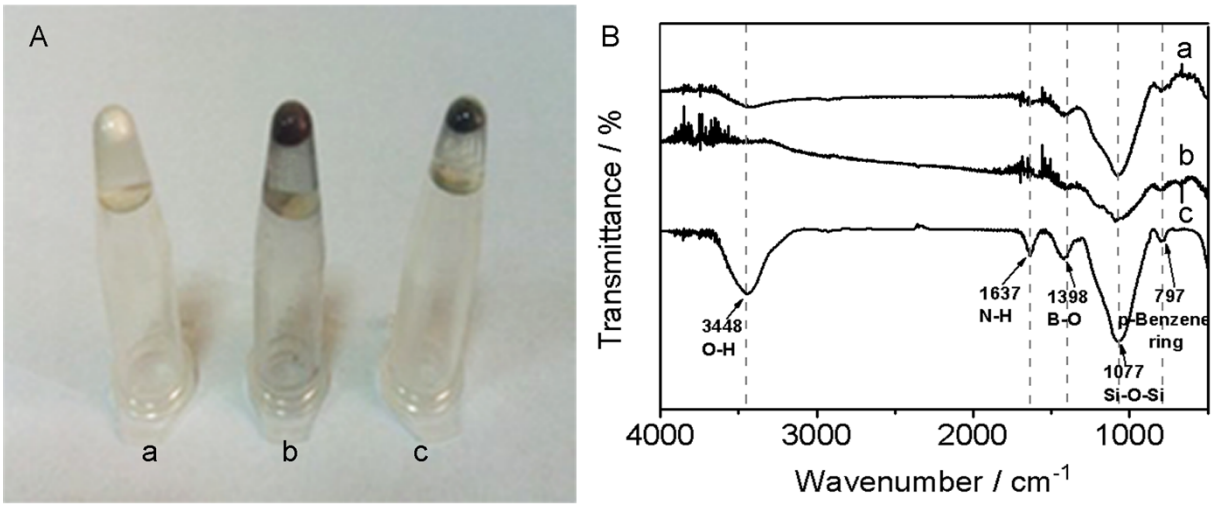

Fig. S1 (A) Photograph and (B) FT-IR spectra of silanized SiBs (a), Au@SiBs (b) and MPB-Au@SiBs (c).

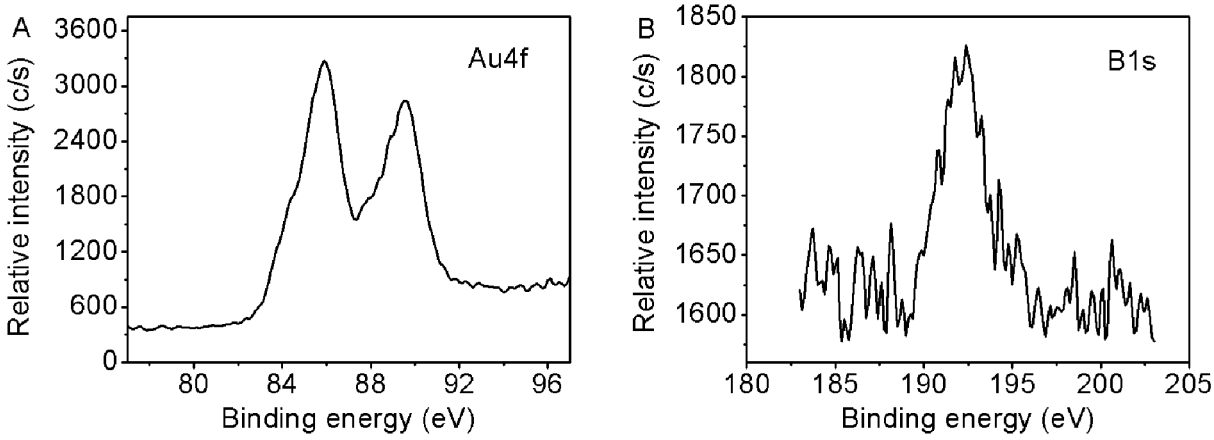

Fig. S2 XPS spectra of (A) Au4f and (B) B1s for MPB-Au@SiBs.

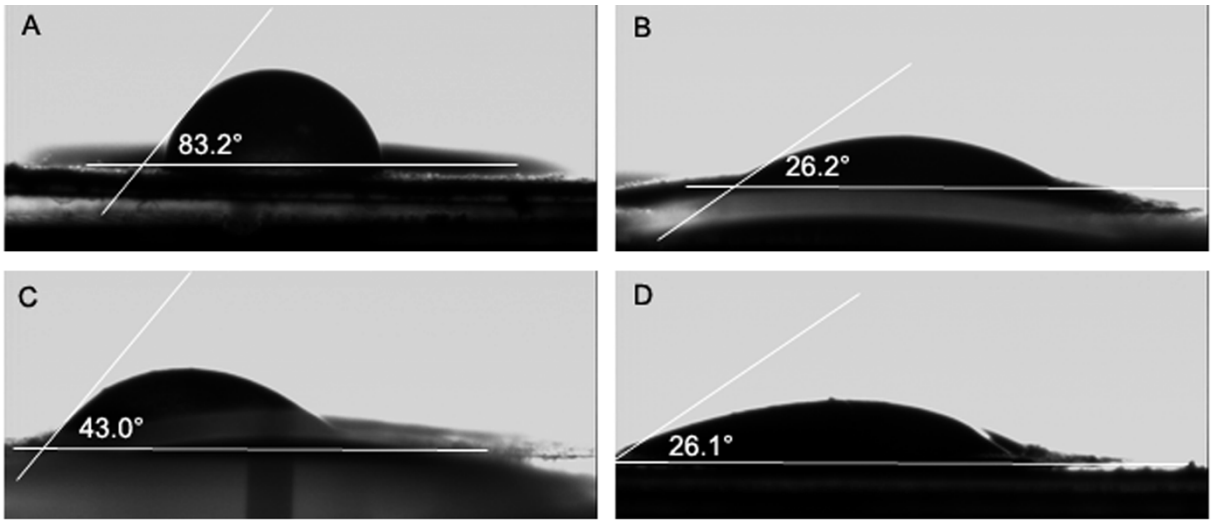

Fig. S3 Contact angles of SiBs (A) before and (B) after treated with APTES, (C) Au@SiBs and (D) MPB-Au@SiBs modified substrates.

Binding capacity of glycopeptides

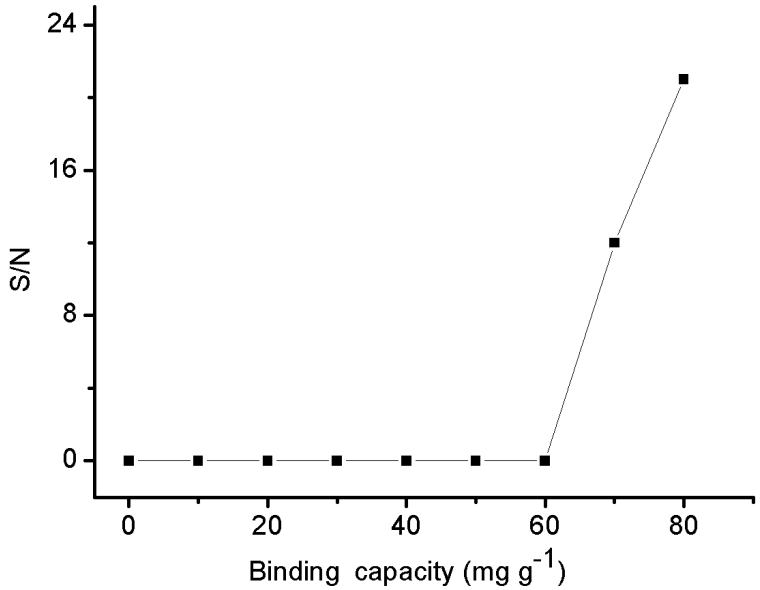

Fig. S4 Binding capacity analysis of glycopeptides on MPB-Au@SiBs by MALDI-TOF MS. The glycopeptides signals were detected from the supernatants of HRP tryptic digests at different concentrations after enriching with MPB-Au@SiBs.

Sensitivity of MPB-Au@SiBs

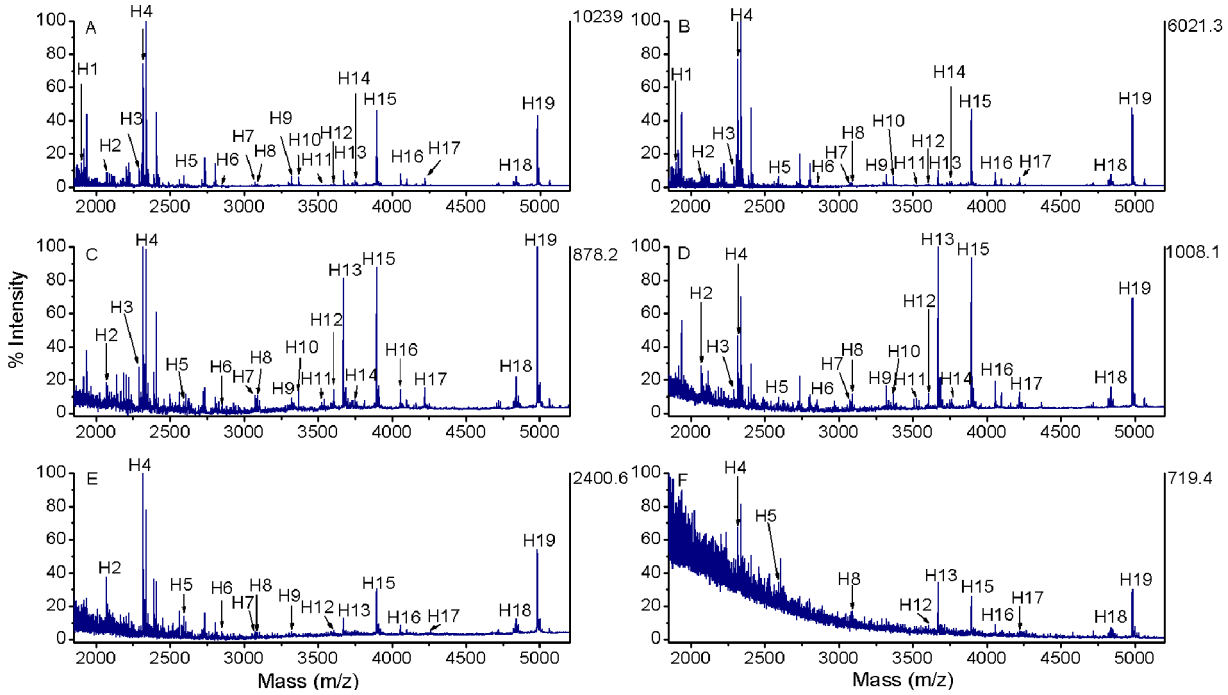

Fig. S5 MALDI mass spectra of the tryptic digests of HRP at (A) 10, (B) 5, (C) 2, (D) 1, (E) 0.2 and (F) 0.1 ng μL^{-1} after enrichment with MPB-Au@SiBs.

Sensitivity of commercial APB agarose

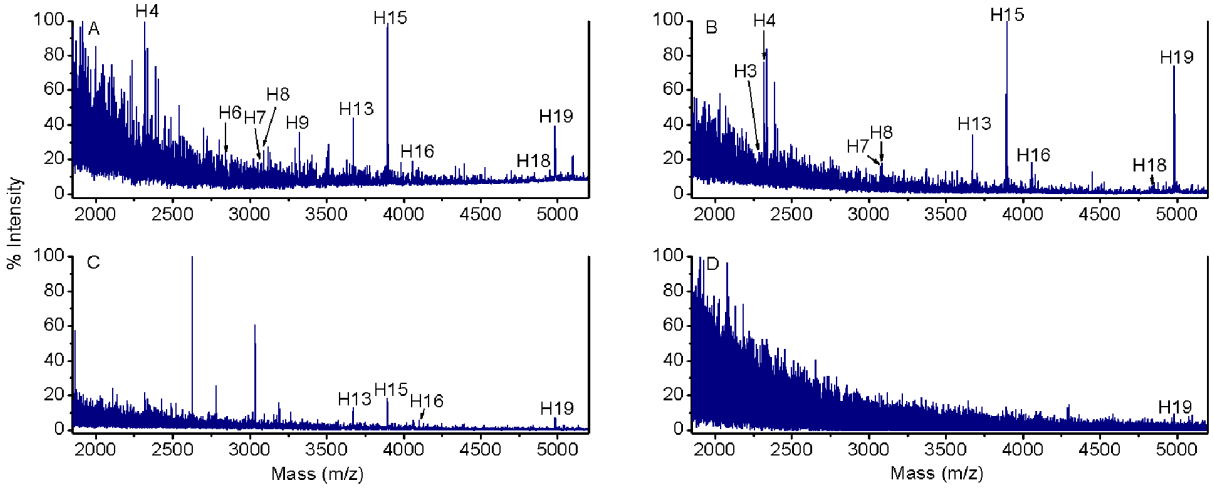

Fig. S6 MALDI mass spectra of the tryptic digests of HRP at (A) 10, (B) 5, (C) 2 and (D) 1 ng μL^{-1} after enrichment with commercial APB agarose.

MS analysis of human IgG tryptic digests

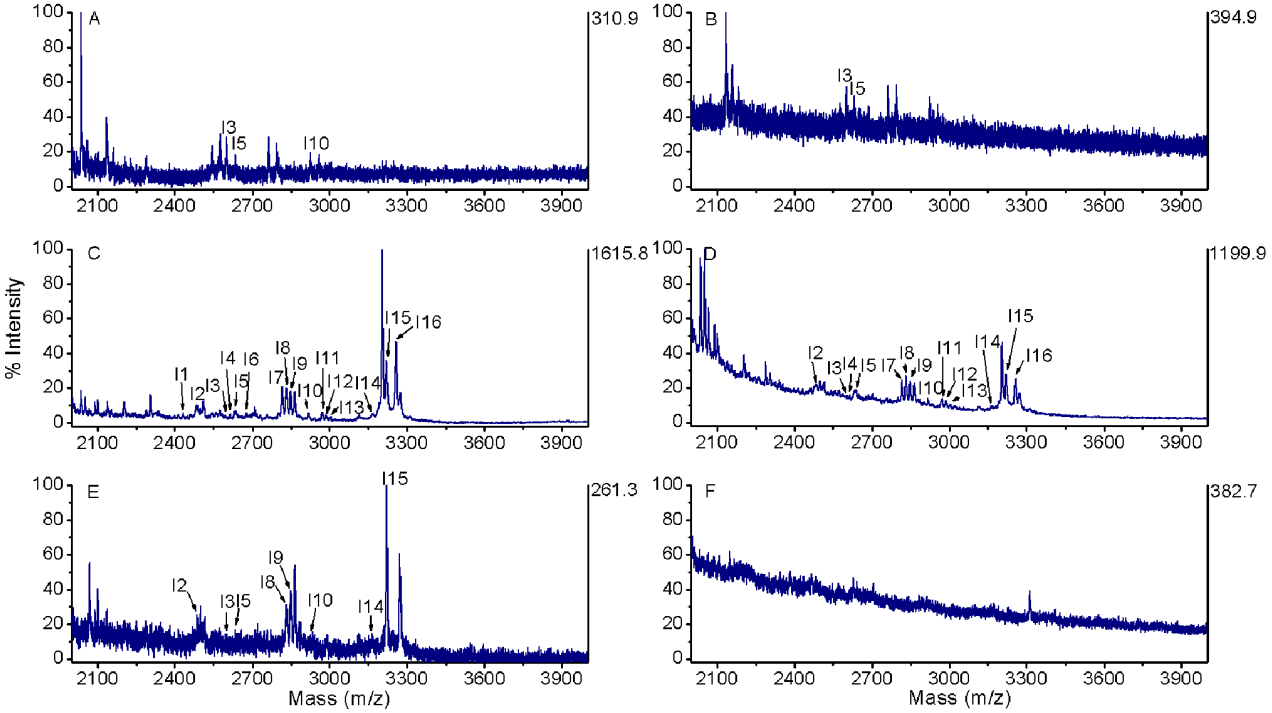

Fig. S7 MALDI mass spectra of the tryptic digests of 5 (A, C, E) and 0.5 (B, D, F) ng μL^{-1} human IgG before (A, B) and after enrichment with MPB-Au@SiBs (C, D) and commercial APB agarose (E, F).

Mechanical stability of the bubbles

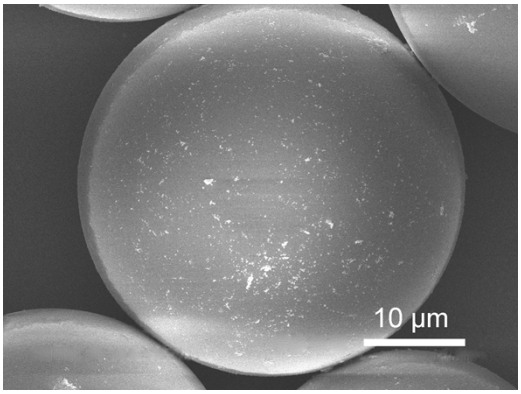

Fig. S8 SEM image of MPB-Au@SiBs after eluting glycopeptides from the surface using the aqueous solution containing 50% acetonitrile and 1% TFA.

Detailed information of glycopeptides

Table S1 Detailed information of the observed glycopeptides in HRP tryptic digest.[S2-S5]

No.	Observed m/z	Glycan composition	Amino acid sequence
H1	1896	XylMan3FucGlcNAc2	N#TTSFR
H2	2068	XylMan3FucGlcNAc2	PN#VSNIVR
H3	2290	XylMan2GlcNAc2	SILLDN#TTSFR
H4	2321	Man2GlcNAc2	MGN#ITPLTGTQGQIR
H5	2591	XylMan3FucGlcNAc2	PTLN#TTYLQTLR
H6	2850	FucGlcNAc	GLIQSDQELFSSPN#ATDTIPLVR
H7	3074	FucGlcNAc	LHFHDCFVNGCDASILLDN#TTSFR
H8	3087	XylMan3FucGlcNAc2	GLCPLNGN#LSALVDFDLR
H9	3323	XylMan3FucGlcNAc2	QLTPTFYDNSCPN#VSNIVR
H10	3353	XylMan3FucGlcNAc2	SFAN#STQTFFNAFVEAMDR
H11	3525	XylMan3GlcNAc2	GLIQSDQELFSSPN#ATDTIPLVR
H12	3606	XylMan3FucGlcNAc2	NQCRGLCPLNGN#LSALVDFDLR
H13	3671	XylMan3FucGlcNAc2	GLIQSDQELFSSPN#ATDTIPLVR
H14	3749	XylMan3GlcNAc2	LHFHDCFVNGCDASILLDN#TTSFR
H15	3895	XylMan3FucGlcNAc2	LHFHDCFVNGCDASILLDN#TTSFR
H16	4058	XylMan3GlcNAc2	QLTPTFYDNSC(AAVESACPR)PN#VSNIVR-H_2O
H17	4223	XylMan3FucGlcNAc2	QLTPTFYDNSC(AAVESACPR)PN#VSNIVR
H18	4837	XylMan3FucGlcNAc2, XylMan3GlcNAc2	LYN#FSNTGLPDPTLN#TTYLQTLR
H19	4983	XylMan3FucGlcNAc2, XylMan3FucGlcNAc2	LYN#FSNTGLPDPTLN#TTYLQTLR

Table S2 Detailed information of the observed glycopeptides in human IgG tryptic digest.[S3,S6]

No.	Observed m/z	Glycan composition	Amino acid sequence
I1	2432	Man3FucGlcNAc3	EEQYN#STYR
I2	2488	Man3GlcNAc4	EEQYN#STYR
I3	2602	Man3FucGlcNAc4	EEQFN#STFR
I4	2618	Man3GlcNAc4Gal	EEQFN#STFR
I5	2636	Man3FucGlcNAc4	EEQYN#STYR
I6	2675	Man3GlcNAc5	EEQFN#STFR
I7	2821	Man3GlcNAc5Gal	EEQFN#STFR
I8	2838	Man3FucGlcNAc5	EEQYN#STYR
I9	2853	Man3GlcNAc5Gal	EEQYN#STYR
I10	2926	Man3FucGlcNAc4Gal2	EEQFN#STFR
I11	2968	Man3FucGlcNAc5Gal	EEQFN#STFR
I12	2983	Man3GlcNAc5Gal2	EEQFN#STFR
I13	3000	Man3FucGlcNAc5Gal	EEQYN#STYR
I14	3161	Man3FucGlcNAc5Gal2	EEQYN#STYR
I15	3219	Man3FucGlcNAc4Gal2Sia	EEQFN#STFR
I16	3251	Man3FucGlcNAc4Gal2Sia	EEQYN#STYR

References

S1. V. L. Schmit, R. Martoglio, B. Scott, A. D. Strickland and K. T. Carron, *J. Am. Chem. Soc.*, 2012, **134**, 59.

S2. Y. Y. Qu, J. X. Liu, K. G. Yang, Z. Liang, L. H. Zhang and Y. K. Zhang, *Chem. Eur. J.*, 2012, **18**, 9056.

S3. Z. C. Xiong, L. Zhao, F. J. Wang, J. Zhu, H. Q. Qin, R. A. Wu, W. B. Zhang and H. F. Zou, *Chem. Commun.*, 2012, **48**, 8138.

S4. L. J. Zhang, Y. W. Xu, H. L. Yao, L. Q. Xie, J. Yao, H. J. Lu and P. Y. Yang, *Chem. Eur. J.*, 2009, **15**, 10158.

S5. D. W. Qi, H. Y. Zhang, J. Tang, C. H. Deng and X. M. Zhang, *J. Phy. Chem. C*, 2010, **114**, 9221.

S6. Z. C. Xiong, H. Q. Qin, H. Wan, G. Huang, Z. Zhang, J. Dong, L. Y. Zhang, W. B. Zhang and H. F. Zou, *Chem. Commun.*, 2013, **49**, 9284.